DEVELOPMENT OF HEALTH CRITERIA FOR SCHOOL SITE RISK ASSESSMENT PURSUANT TO HEALTH AND SAFETY CODE SECTION 901(g):

PROPOSED CHILD-SPECIFIC REFERENCE DOSE (chRD) FOR SCHOOL SITE RISK ASSESSMENT-

Endosulfan

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PROGRAM INTRODUCTION

Implementation of Health and Safety Code (HSC), Section 901(g)

Health and Safety Code (HSC), Section 901(g), requires the Office of Environmental Health Hazard Assessment (OEHHA), in consultation with the appropriate entities within the California Environmental Protection Agency, to identify those chemical contaminants commonly found at school sites and determined by OEHHA to be of greatest concern based on child-specific physiological sensitivities. HSC 901(g) also requires OEHHA to annually evaluate and publish, as appropriate, numerical health guidance values (HGVs) for five of those chemical contaminants until the contaminants identified have been exhausted. HGVs established by this mandate are intended for use in the assessment of risk at proposed or existing California school sites. At this time, OEHHA focuses its evaluation on non-cancer effects of the identified chemicals, pending the completion of a new method for developing HGVs based on child-specific carcinogenic effects. Accordingly, current HGVs are in the form of a child-specific reference dose (chRD) or child-specific reference concentration (chRC).

This chapter serves as a background for the technical chRD or chRC reports. For those that are not familiar with this OEHHA program, it is advisable to review this chapter prior to reading the individual chRD reports.

Developing a chRD or chRC

Challenge

The use of appropriate HGVs and exposure parameters is essential to provide an unbiased assessment of the health risk at an existing or a proposed school site. Since school children have higher air, food and water intake relative to their body weight compared to adults; and have activity or behavioral patterns that may lead to higher exposure to environmental contaminants than adults, these higher intakes and unique activity patterns need to be considered in developing a set of child-specific exposure parameters for use in the risk assessment. OEHHA has analyzed these exposure parameters in issuing the report, Guidance for Assessing Exposures and Health Risks at Existing and Proposed School Sites (http://www.oehha.ca.gov/public_info/public/kids/pdf/SchoolscreenFinal.pdf).

With respect to evaluating non-cancer risk by comparing the potential chemical exposure against the corresponding health criteria in the school setting, HGVs in the form of child-specific reference doses or concentrations should be used. Until the inception of the HSC 901(g) program, these child-specific HGVs were not available. For most part existing reference doses or concentrations for non-cancer endpoints, which were based on adult human or animal data, were used. The Food Quality and Protection Act of 1996 (http://www.epa.gov/opppsps1/fqpa/) was the first attempt to create a child-specific HGV (Bigsby, et al, 1999). It mandated a safety factor of 10 unless data existed to indicate that children were not more sensitive than adults. However, some scientists (Bruckner, 2000; Renwick, 2000; Pelakis et al. 2001; Dourson, 2002;) have reasoned that the intraspecies uncertainty factor of 10, the default factor, will adequately protect children because it was designed to account for pharmacokinetic differences brought about by

metabolizing isoenzyme variations, differences in metabolism and kinetics during pregnancy, renal differences during chronic illnesses, and age-produced variations from birth to elderly.

A case can be made for the development and application of child-specific HGVs based on studies in children or young animals rather than relying solely on a safety factor or uncertainty factor. While locating the appropriate data is a challenge, OEHHA has strived to do so because children can be more (or less) susceptible to chemical effects due to pharmacokinetic and pharmacodynamic differences between them and adults, and thus empirical data in the young would be preferable. Vulnerability often depends on the organ system in question and its developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life, including adolescence. During its critical period(s), a particular structure or function is most sensitive to disruption due to interactions between a toxicant and target tissues that are undergoing biochemical changes. Damage may not be evident until a later stage of development (DeRosa et al, 1998; Bigsby et al, 1999). The brain, for example, is an organ with distinct neurodevelopmental stages that occur in temporally distinct time frames across different regions, so the specific chemical, dose, and time of exposure during development determine if a specific function in the brain will be altered (Faustman et al, 2000).

Differences also exist between children and adults with respect to their absorption, distribution, metabolism, and elimination of chemical contaminants. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al, 1980; NRC, 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al, 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water, and their brains and livers are proportionately larger (Altman PL, 1974; Fomon, 1966; Fomon et al, 1982; Owen G.M., 1966; Widdowson E.M., 1964). The infant also has an immature blood-brain barrier (Adinolfi, 1985; Johanson, 1980)and probably an immature blood-testis barrier (Setchell B.P, 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al, 1990; Leeder and Kearns, 1997; NRC, 1993; Vieira et al, 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns, who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman PL, 1974; NRC, 1993; West J.R., 1948). Children and adults may differ in their capacity to repair damage from chemical insults.

OEHHA faces an additional challenge when evaluating chemicals that are potential endocrine disruptors. The topic of endocrine disruption during development has been the subject of much scientific and regulatory discussion (Colborn et al,1993a; Colborn et al, 1993b; Cranmer et al, 1984; US EPA, 1998). While not all chemicals selected for the OEHHA review are endocrine disruptors, the endocrine disruptors do pose a greater concern because not only could they directly impact the maturation and proper functioning of the endocrine system, they could also interfere with hormonal signal transduction that leads to abnormal growth and functioning of other target organs (e.g., immune and nervous systems) in school children. Exposure to

endocrine disruptors during critical "programming" periods in development, in contrast to exposure during adulthood, may produce irreversible effects on the reproductive, nervous, and/or immune systems (Bigsby et al, 1999). In adulthood, these endocrine disruptors might only produce reversible effects by participating in the "seesaw" process of stimulation and feedback inhibition.

Given the complexity of hormone signaling processes, it is also not surprising to find the evaluation of the dose and response relationship to be another challenge. The shape of the dose response curve may not be linear, but rather shaped like an upright U or an inverted U (Markowski et al, 2001; vom Saal et al, 1997). This makes data interpretation difficult when the study does not include sufficient treatment doses to span the entire range of interest.

U.S. EPA and the March of Dimes sponsored a workshop -- Identifying Critical Windows of Exposure for Children's Health -- in September 1999 to systematically review the state of knowledge on prenatal and postnatal exposures and subsequent outcomes (Selevan et al, 2000). The workshop focused on the nervous, immune, respiratory, reproductive, and endocrine systems—organ systems that are still undergoing development and maturation in children and thus deemed to be highly vulnerable to chemical insults. Workshop participants noted that data pertaining to children's sensitivities to environmental contaminants during various critical developmental periods are limited. In particular, little attention has been given to studying peripubertal/adolescent exposures or adult consequences from childhood exposure. Thus, the state of scientific knowledge pertaining to chemical effects on children is and will continue to be a limiting factor in OEHHA's ability to develop child-specific HGVs for these contaminants.

In summary, with rare exceptions the use of a study in children or young animals as the basis for a child-specific HGV is preferred, even when studies in adult humans or animals encompassing a greater dose range or a larger experimental population exist and a biological mechanism of action can be established from corroborating studies. If a study in the young does not exist, the challenge is to integrate studies supporting a biological mechanism for greater sensitivity in the young with studies on adults to justify the application of appropriate safety factors.

Process

In June 2002, OEHHA issued a report, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code, Section 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites," documenting the process by which OEHHA identifies chemicals and presenting a compilation of 78 chemicals. The report can be found at http://www.oehha.ca.gov/public_info/public/kids/schoolsrisk.html. The compilation, whose sole purpose is to provide OEHHA staff with a manageable list of chemicals to work from, has no regulatory status and is a living document – chemicals may be added or removed as new information becomes available.

The chRD development process begins with the prioritization of chemicals from the compilation described in the June 2002 report. OEHHA has employed the following criteria, recognizing that

often the availability of health effect data may be the overriding consideration in the selection of chemicals for evaluation.

1. Chemicals having a strong indication of their presence at school sites according to monitoring studies or other reliable sources.

2. Chemicals cited to have possible adverse effects in three or more of the systems that are undergoing critical development during childhood: the nervous, immune, respiratory, reproductive, or endocrine systems.

3. Chemicals that other OEHHA programs have identified as a concern.

From a public health protection standpoint, the OEHHA scientists working on health guidance values for children as mandated by Health & Safety Code 901(g) have adopted the following procedures in developing chRDs or chRCs. First, in order to protect children from infancy through the time they leave school, chRDs must consider school-aged children up to age 18, and infants and toddlers in daycare facilities located at school sites. Second, OEHHA opts to consider the most sensitive species and endpoints in our evaluations, meaning that the lowest Lowest-Observed-Adverse-Effect-Level (LOAEL) or No-Observed-Adverse-Effect-Level (NOAEL) from available literature, preferably an effect on a developing organ system, would be selected. Third, the paucity of data has underscored the reality that the databases for sensitive endpoints may be incomplete. An uncertainty factor for database deficiency will be considered as appropriate. Fourth, because quantifying differences in susceptibility between a developing organ system and a mature one are hampered by the availability of studies that intentionally compare an effect in young animals with one in adult animals and available data are mainly from developmental toxicity studies that limit dosing to the mother during pregnancy, OEHHA staff have decided that these studies can be used for development of a child-specific health guidance value (chRD or chRC) if it is reasonable to assume that the effect of the chemical on the target organ in the offspring animal would likely occur on the same target organ undergoing development after birth in humans. If studies that include gestational dosing of the mother and lactational dosing of the pups (a protocol of the U.S. EPA Developmental Neurotoxicity Health Effects Test) are available, OEHHA will also consider these studies acceptable for establishing a chRD or chRC if the development of the critical organ system continues to occur during childhood.

Finally, these prenatal and perinatal studies are frequently part of a series of studies to elucidate a "mechanism of toxicity." These studies may not have used a large number of animals or dose ranges. However, due to the critical windows in which cell proliferation and differentiation are occurring in specific organ systems during childhood, a study in young animals is usually preferred over one in adults, even adult humans. With corroborating studies showing a mechanism of action and biological plausibility, OEHHA will consider using these studies as appropriate. However, in rare cases, data from adult animals may be used, if they are from high quality studies and if there are data to provide a means of inference to critical windows of development in young animals.

Status

In March 2003, OEHHA issued a draft report proposing chRDs for the first five evaluated chemicals: Cadmium, Chlordane, Heptachlor/Heptachlor Epoxide, Methoxychlor, and Nickel, which be found at: http://www.oehha.ca.gov/public_info/public/kids/schools603.html.

In the current cycle, OEHHA selected 19 chemicals for which literature searches were performed. These chemicals included endosulfan, manganese, pentachlorophenol, toluene, lead, arsenic, aldrin, atrazine, DDE, DDT, dieldrin, endrin, hexachlorobenzene, lindane, malathion, perchloroethylene, permethrin, selenium, and trichloroethylene. The Public Health Library at the University of California at Berkeley assisted in literature search. OEHHA, in turn, reviewed the citations and abstracts, and evaluated relevant qualitative papers and quantitative studies.

As a result, OEHHA is establishing a chRD for endosulfan, manganese, pentachlorophenol, toluene, and lead. This chapter serves as a background for the individual chRD reports.

With respect to arsenic, aldrin, atrazine, DDE, DDT, dieldrin, endrin, hexachlorobenzene, lindane, malathion, perchloroethylene, permethrin, selenium, and trichloroethylene, qualitative data indicate that they may adversely impact school children by affecting one or more developing organ systems (endocrine, nervous, immune, reproductive, or respiratory). However, these mechanistic studies are usually conducted at a higher dose range, rendering them less useful in the chRD development process. As part of this public comment process, OEHHA is seeking public input to identify relevant quantitative studies that may be used to derive a LOAEL or NOAEL from which to develop a chRD for these chemicals.

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ENDOSULFAN

Orientation to the document

This report on the establishment of a child-specific reference dose (chRD) for endosulfan has been divided into sections in order to try to provide information to fit the needs of two audiences. The "Rationale for Establishing a Child-Specific Reference Dose (chRD) for Endosulfan," is written to answer questions that a children's health professional may have about the reasons for creation of a chRD for children at school sites. It provides a narrative in the form of answers to questions similar to those done by the Agency for Toxic Substances and Disease Registry (ATSDR) in their Public Health Statement. "Details of the Scientific Evidence for Establishment of a Child-Specific Reference Dose (chRD) for Endosulfan" presents the detailed discussion of the studies that OEHHA scientists considered significant and the scientific rationale for choosing particular studies. It has been placed in a separate section because it may not be of interest to all readers.

Rationale for Establishing a Child-Specific Reference Dose (chRD) for Endosulfan

What is endosulfan?

Endosulfan is a broad-spectrum organochlorine pesticide. The organochlorine insecticides are a diverse group of agents belonging to three chemical classes according to their structure: DDT is in the dichlorodiphenylethaneone class with DDD, dicofol, perthane, methoxychlor, and methlochlor; endosulfan is a cyclodiene with aldrin, dieldrin, heptachlor, and chlordane; and the chlorinated benzenes contain lindane hexachlorobenzene, and hexachlorohexane. From the mid 1940s to the 1960s these agents were used extensively to control pests on crops, forests, structures, and on humans. Endosulfan is the only one in its class that is still registered for use. It kills insects, mites and ticks on contact by blocking chloride channels in invertebrate nerves. It is currently registered for use on a variety of vegetables, fruits, cereal grains, and cotton, as well as ornamental shrubs, trees, vines, and ornamentals in commercial agricultural settings, such as greenhouses. It is not sold as a retail product to individuals and is only applied by licensed applicators. However, it was one of the top 100 pesticides used in California in 2001, as 151,000 pounds were used to treat 162,000 acres, according to the California Department of Pesticide Regulation.

How is Endosulfan's use as a pesticide regulated?

Endosulfan was first registered as a pesticide in the United States in 1954. The 1988 amendments to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) required U.S. EPA to review the health and environmental effects of all pesticides registered before November 1, 1984. FIFRA as amended in 1996 by the Food Quality Protection Act (FQPA) requires that all pesticides meet new safety standards. EPA must be able to conclude with "reasonable

certainty" that "no harm" will come to infants, children, or other sensitive individuals exposed to pesticides.

Endosulfan has been undergoing review for reregistration, a process in which the registrant submits to U.S. EPA toxicity data relating to human health and ecological effects for the pesticide's active ingredients. The data from toxicity tests are reviewed for completeness and reassessment of tolerances (pesticide residue limits in food) to meet the safety standard established by the FQPA, which provides an extra 10-fold safety factor for children unless there are data to demonstrate that children are not more sensitive than adults. More data on Pesticide Reregistration can be found at http://www.epa.gov/oppfead1/trac/factshee.htm.

U.S. EPA reported in its document, *Reregistration Eligibility Decision for Endosulfan (RED)*, November 2002, that endosulfan was eligible for reregistration if the registrant performed a series of toxicology studies, the results supported registration, and new usage restrictions to reduce human and environmental exposure were followed (U.S. EPA, 2002). The requested studies included 13 ecological toxicity studies, two surface and groundwater monitoring studies, six studies relating to application procedures, a 90-day study to investigate neurotoxicity in adult rats, and a study to investigate neurotoxicity from gestational exposure in rats. Details can be found in the document, which is available online at http://www.epa.gov/REDs/endosulfan_red.pdf. While the studies are being conducted, the use of endosulfan can continue if mitigation procedures to reduce dietary, worker and ecological risks are carried out.

Research has also continued on the mechanism of insecticidal activity of endosulfan, other chemically similar cyclodienes, and the development of insect resistance. The established target sites are membrane-bound proteins containing chloride ion (Cl-) channels, specifically the $GABA_A$ receptor/Cl ionophore complex (Bloomquist 2003). An altered GABA receptor is responsible for cyclodiene resistance (Ffrench-Constant et al., 2000). Endosulfan binds at the noncompetitive blocker site, blocking the GABA-stimulated chloride flux that inhibits nerve conductance (Kamijima and Casida, 2000; Bloomquist, 2003).

What characteristics make endosulfan of concern in the environment of schools?

Endosulfan presents a concern for human and environmental ecosystems because it is persistent in soils that were formerly agricultural land, where many new schools are being constructed, and it is capable of moving from the point of application to areas where it is not used. New developments are being constructed in agricultural areas where endosulfan is still used, and endosulfan can move to locations far distant from the point of application, such as the Arctic and national parks. It persists in soils, although not as long as other chlorinated pesticides such as DDT. Endosulfan and one of its transformation products, endosulfan sulfate, have been reported to have an environmental half-life ranging from nine months to six years, depending on specific conditions (U.S. EPA, 1999).

The technical grade of endosulfan contains a mixture of 70 percent alpha and 30 percent beta endosulfan, both of which are biologically active. The different properties of the two isomers give endosulfan the ability to adhere to soil, volatilize into air, and move as particulates or dust

in air or water. The beta isomer is generally more persistent and the alpha isomer is more volatile. Both isomers have a high affinity for sorption onto soils. Both isomers are degraded by hydrolysis in alkaline water but both are stable to hydrolysis in acidic waters, where microbial metabolism is the predominant route of degradation (U.S. EPA, 1999).

The major transformation products are endosulfan diol from hydrolysis and endosulfan sulfate from soil metabolism. These compounds have insecticidal activity similar to the parent compound and are also of toxicological concern (U.S. EPA, 1999).

A database named @Risk, created by the Environmental Working Group (http://www.ewg.org/california/@risk/1998ca15.php), based on a computer analysis of state pesticide use data, indicates that endosulfan was used within 1.5 miles of a number of rural county schools in 1995, the last year for which data were released. A recent investigation by OEHHA of metals in soil outside on the school site compared to metals in floor dust inside the classroom has revealed that a greater concentration exists inside the classroom. This indicates that chemicals in school-site soil, such as pesticides like endosulfan, can be tracked inside the classroom to expose children at higher concentrations than are found outside (TRI, Inc et al, 2003).

Why has OEHHA investigated the toxicity of endosulfan to children?

OEHHA included endosulfan in the "Compilation of Chemicals Potentially Found at School Sites" because 1) the California Department of Toxic Substances Control has reported that it is present at proposed school sites and 2) review articles described endosulfan as a male reproductive toxicant and a neurobehavioral toxicant due to the alteration of endocrine hormone balance during development, a phenomenon known as endocrine disruption (Bernstein, 1984; Brucker-Davis, 1998; Olea et al, 1998). Furthermore, it is one of the persistent and abundant chemicals in our environment that U.S. EPA concluded would be present in human blood and urine, as well as air and water around humans, and so U.S. EPA included it in the National Human Exposure Survey (NHEXAS). More information on the compilation of chemicals that are potentially present at school sites can be found in the document entitled, "Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code 901(g): Identification of Potential Chemical Contaminants of Concern at California School Sites, June 2002" on the OEHHA website at http://www.oehha.ca.gov/public_info/public/kids/pdf/ChildHealthreport60702.pdf.

Endosulfan exposure is of special concern for children's health because, as U.S. EPA noted in its reregistration document, its review found evidence that endosulfan is an endocrine disruptor (U.S. EPA, 2002). Endocrine disruptors are chemicals that mimic or antagonize the female and male sex hormones, estrogens and androgens, as well as thyroid hormones, T_3 and T_4, and the endogenous chemicals that may antagonize hormones. Endocrine disruptors, also called endocrine modulators, can alter the normal balance of hormones at critical points in development, and thus disrupt processes or tissues these hormones affect. Sex steroid hormones affect the male and female reproductive organs, the central nervous system, and the immune system. (U.S. EPA, 2002; Colborn et al, 1993; Bigsby et al, 1999). The thyroid hormones affect

most tissues. Exposure to endocrine disruptors is of particular concern for children because disruption of the action of estrogen and androgen during critical periods of development can lead to permanent alterations in the reproductive, nervous, and immune systems during growth from before birth through adolescence (Bigsby et al, 1999).

Adverse health effects in children were noted in a 2001 study by the National Institute of Occupational Health (NIOH). These children were from a village in the state of Kerala, India, which is located downstream from cashew tree orchards that receive repeated aerial spraying of endosulfan. These adverse health effects included a higher prevalence of learning disabilities, low intelligence quotient (IQ) and scholastic backwardness. A published article in Environmental Health Perspectives on the same study (Saiyed et al, 2003) reported that male schoolchildren in the village where endosulfan had been sprayed manifested a delay in onset and progress through puberty compared to those in an unexposed community. An increased number of congenital abnormalities related to testicular descent were also observed. Some controversy has insued as industry spokesmen disagreed with the likelihood of only exposure to endosulfan, especially from water (Abraham, 2004; Indulkar, 2004). Saiyed (2004) responded that sampling had been done at various times after spraying and endosulfan adsorbs to soil particulates. More details can be found in the section on "Scientific Details of Studies to Establish a chRD for Endosulfan".

What are the existing Health Guidance values developed by other agencies?

The U.S. EPA oral Reference Dose (RfD), or safe dose for oral exposure, is 6×10^{-3} mg/kg-day, based on the observation that 15 ppm in the feed (equivalent to 0.6 mg/kg-day in males) is a "no observable adverse effect level" (NOAEL) in a 2-year adult rat feeding study by the manufacturer, Hoechst Celanese Corp., (U.S. EPA, 1999). In this type of study, exposure began when the rats attained adulthood and continued for life. The "lowest observed adverse effect level" (LOAEL) was 75 ppm in the feed (equivalent to 2.9 mg/kg-day for male and 3.8 mg/kg-day for females) based on reduced body weight gain in both sexes and increased incidence of progressive glomerulonephrosis (kidney disease) and blood vessel aneurysms (weakening of the vessel's wall) in adult males.

U.S. EPA also considered a one-year dog feeding study with a NOAEL of 10 ppm in the feed (equivalent to 0.57 mg/kg-day in females). The LOAEL was 30 ppm in the feed based on decreased weight gain in males and neurologic abnormalities in both sexes.

The EPA reported that no evidence of reproductive toxicity was found in a two-generation reproduction study in an infrequently used rat strain, Crl:COBS CD(SD)BR. However, endosulfan caused increased pituitary and uterine weights in this study (U.S. EPA, 1999a; http://www.epa.gov/iris/subst/0235.htm). The endpoints in a two-generation reproduction study are the ability of the first generation (F_1) to breed and produce F_2 young which appear healthy, despite the fact that the F_0 (grandparents) were dosed before conception of progeny through lactation of their F_1 offspring, and the F_1 generation, which became parents of the F_2 generation, had been exposed from their own gestation through conception and lactation of the F_2 pups. Since no reproductive deficits were reported for the registrant's study, the NOAEL for

reproductive toxicity is considered to be equal to or greater than the highest dosage tested, 75 ppm in feed (equivalent to 5.4 mg/kg-day in males; and 6.6 mg/kg-day in females).

U.S. EPA noted that a rat Developmental Neurotoxicity study had not been done and it constituted a gap in the data record when the Reference Dose (RfD) was established. The U.S. EPA review for re-registration concluded, therefore, that, under the Food Quality Protection Act (FQPA), a safety factor of 10X to protect children should be retained for endosulfan when considering intake from food (U.S. EPA, 2002). It should be noted that the requirements of the FQPA do not apply to endosulfan in soil when the Department of Toxic Substances Control (DTSC) of Cal/EPA considers safe levels for clean-up of potential school sites. The DTSC uses U.S. EPA RfDs and carcinogen slopes in risk assessments for school sites unless a chRD has been established.

Why did OEHHA decide to establish a child-specific reference dose (chRD) for endosulfan?

US EPA's reference dose for endosulfan is based on experiments in adult animals. The U.S. EPA chose to keep the 10X safety factor to protect children from exposure to pesticides in food when allowing endosulfan to be eligible for reregistration. But, the 10X factor for food is an additional safety factor added onto existing safety factors from a LOAEL or NOAEL of a critical effect in an adult animal study. OEHHA staff were concerned that California school children would not be protected by the U.S. EPA RfD which applies to school site soil contaminants and does not incorporate the FQPA safety factor of 10X which applies only to pesticides on foods. In its decision to consider reregistration of endosulfan, the U.S. EPA reported that the data submitted by the registrant contained no reliable data to address concerns or uncertainties regarding (1) evidence for increased susceptibility of young rats, (2) additional evidence for endocrine disruption, (3) the neuroendocrine effects in the young, and (4) the lack of a developmental neurotoxicity study (DNT) (U.S. EPA, 2002). However, toxicity studies reported in the scientific literature indicated that prenatal/perinatal and pubertal exposure to endosulfan is toxic to multiple organ systems, which is consistent with endocrine disruption during development of critical organ systems. Endosulfan suppresses the humoral (IgG and IgM) and cell mediated immune responses in pubertal animals fed very low doses (Banerjee and Hussain, 1987; Banerjee and Hussain 1986). Endosulfan affects the differentiation of the testis and production of sperm in young male rats (Sinha et al, 1997a; Chitra et al, 1999; Sinha et al, 2001). Prenatal and postnatal exposure to endosulfan impairs learning (Paul et al, 1994; Chitra et al, 1999; Sinha et al, 2001), and alters behavior influenced by neurotransmitters and neuroendocrine pathways (Paul et al, 1993; Zaidi et al, 1985).

The mechanism of toxicity of endosulfan in insects, blocking of $GABA_A$ receptors for chloride channels in neurons (Ratra et al, 2001; Bloomquist, 2003), can produce toxicity in mammals (Lawrence and Casida, 1984; Bloomquist, 2003; Ratra and Casida, 2002) because $GABA_A$ receptors are found in the brain and at a similar concentration in peripheral tissues, such as pancreatic islet cells, the oviduct, the myenteric plexus of the gut, adrenal chromaffin cells (Iwasa et al, 1998) and recently in the testis and sperm (Hu and Yan, 2002; Geirgerseder et al, 2003; He et al, 2003; Li et al, 2005).

GABA is known as an inhibitory neurotransmitter in mature brain, and as an excitatory neurotransmitter during brain development when it regulates morphogenesis of brain structures in the immature central nervous system (Takayama and Inoue, 2004). GABA is excitatory in

immature neurons because the concentration of chloride ion in the neuron is high, and when the $GABA_A$ receptor gate in the cell membrane opens, the chloride ion concentration decreases, depolarizing the neuron and creating an action potential that propagates down its axon. In mature neurons, the concentration of chloride ion in the neuron is low (an equilibrium potential of -68mV), and when the $GABA_A$ receptor gate opens, chloride ions flow into the neuron to hyperpolarize it, inhibiting the generation of an action potential (Takayama and Inoue, 2004).

$GABA_A$ receptors are found in rat interstitial testicular cells, most of which are testosterone-producing Leydig cells (Geigerseder et al, 2003) and spermatozoa (Hu et al, 2002; Li et al, 2005). If endosulfan blocks the GABA receptors for chloride channels, many aspects of spermatogenesis would also be blocked, such as regulation of endocrine function in the testis through Leydig cells, maturation and differentiation of germ cells and initiation of cell proliferation and differentiation in the interstitial testicular compartment (Giergerseder et al, 2003). In addition, GABA receptors on sperm play a role in triggering the acrosome reaction whereby the sperm become active (Ritta et al, 1998; Hu and Yan, 2002; He et al, 2003).

Endosulfan has induced oxidative stress in Saccharomyces cerevisiae and human cell cultures (Sohn et al, 2004). Reactive oxygen species (ROS), such as hydrogen peroxide, produce oxidative stress and cause a significant increase in intracellular chloride (hyperpolarization) through GABA-gated chloride channels in brain slices (Sah et al, 2002). Low doses of endosulfan created moderate levels of ROS in a human leukemic cell line (Kannan et al, 2000) and produced a form of programmed cell death called apoptosis. The generation of ROS may occur by uncoupling oxidative phosphorylation (Kannan et al, 2000) and reducing $GABA_A$-gated chloride channel function.

Thus a possible mode of action is that endosulfan can uncouple oxidative phosphorylation (Kannan et al, 2000) to create ROS and oxidative stress (Sohn et al, 2004) which can produce apoptosis in immune cells (Kannan et al, 2000), testicular damage (Rao et al, 2005) and neuronal damage (Sah et al, 2002). These mode of action studies may explain the results of the in vivo studies demonstrating testicular damage (Sinha et al, 1997a; Chitra et al, 1999; Sinha et al, 2001), neurobehavioral disruptions (Zaidi et al, 1985; Seth et al, 1986; Paul et al, 1992;1993;1994), and immune toxicity (Banerjee and Hussain, 1986; 1987) in young rodents. Effects on these organ systems are consistent with endocrine disrupting activity through inhibition of cellular respiration and/or antagonism of GABA receptors for chloride channels that leads to alterations in development in the young. Summaries of all animal studies that OEHHA considers pertinent can be found in Table 1 and Table 2 in the next section, "Details of the Scientific Studies that OEHHA Considered in Developing a Child-Specific Reference Dose (chRD) for Endosulfan".

OEHHA also noted that the two-generation reproduction study submitted for reregistration to determine if endosulfan had reproductive or fertility effects would not have been able to detect the adverse effects reported in rats in the peer-reviewed scientific literature. The study submitted by the registrants appears to have been done following the 1984 protocol, rather than the one contained in the 1998 Health Effects Test Guidelines, Office of Pollution Prevention and Toxic Substances, OPPTS 870.3800, Reproduction and Fertility Effects. The 1998 protocol requires data to document that planned doses to animals were achieved, male and female reproductive

organs to be examined microscopically even in the absence of visible abnormalities, and sperm counts from one testicle be done. Furthermore, strains of rats used for laboratory studies were selected for high fecundity and are known to have very high sperm counts, so conception has been observed to occur even if dosing caused more than an 63 percent reduction in sperm count (Aafjes et al, 1980). In addition, neither the two-generation reproduction study guidelines from 1984 nor those from 1998 require assay of any neurobehavioral endpoints in the offspring, so these effects of endocrine disruptors would not be detected. A developmental neurotoxicity study was not required when endosulfan was first registered, so U.S. EPA requested that the Developmental Neurotoxicity protocol be conducted.

What studies demonstrate a critical effect of endosulfan in children?

The weight of evidence from all the available scientific literature indicates that endosulfan is an endocrine disruptor which can affect critical development of the immune, reproductive and neural systems. Development of the male reproductive system appears to be the most sensitive effect that has the best quantitative data to date. OEHHA calculated a child-specific chRD from a study by Chitra, et al. (1999) in which endosulfan adversely affected the male reproductive system in pubertal rats. The studies in Table 1 corroborate the toxicity to the male reproductive system in a variety of different biochemical and morphological assays.

In the Chitra et al. study (1999) the pubertal rats were given 1 mg/kg-day of endosulfan by mouth for 30 days and were sacrificed 24 hours after the last treatment. Endosulfan at this dose caused a reduction in body weight and decreased the weights of the testes, epididymis, seminal vesicles, and ventral prostate. The authors also investigated biochemical enzymes and metabolic energy sources that are associated with spermatogenesis, and which may be decreased by antiandrogens to lead to decreased spermatogenesis. Endosulfan decreased the rat's testicular lactate and pyruvate, both of which are critical for spermatogenesis. More details on these energy substrates in testis are available in the section, "Scientific Details of Studies to Establish a chRD for Endosulfan."

Chitra and colleagues (1999) showed that endosulfan exposure decreased the specific activity of testicular 3β-hydroxysteroid dehydrogenase. This enzyme, along with 17β-hydroxysteroid dehydrogenase, is essential in the synthesis of testosterone in Leydig cells. Testosterone stimulates germ cell proliferation, the initiating step in spermatogenesis. It follows that testicular DNA and RNA would decrease if spermatogenesis were decreased, which was observed by (Chitra et al, 1999b).

Jaiswal and colleagues (2005) described histopathological changes in seminiferous tubules after 7.5 mg/kg-bw endosulfan: a decrease in Sertoli cells, anucleular cells, only scattered Leydig cells, and destruction and disappearance of most spermatids and spermatozoa . Endogenous antioxidants and liver superoxide dismutase (SOD) decreased and catalase increased after 1 mg/kgday of endosulfan as a whole body aerosol for 30 days (Bebe and Panemangalore, 2003). An exogenous antioxidant, 5-aminosalicyclic acid (5-ASA), can prevent or ameliorate cell destruction and death if it is given after endosulfan or simultaneously with endosulfan exposure

for 10 days to pubertal rats. 5-ASA is an antioxidant which scavages free radicals produced by lipid peroxidation in cellular membranes by endosulfan,

OEHHA believes that the human study by Saiyed et al. (2003), the studies in rats during gestation and after weaning by Sinha et al. (1997 and 2001), and the study by Chitra et al. (1999), in pubertal rats corroborate each other and indicate that male reproduction is the most sensitive organ system that endosulfan affects. The factors analyzed in the rat male reproductive toxicity studies are displayed and compared in Table 1. It should be noted that these studies were only of short duration (4 to 12 weeks), rather than chronic studies. Studies which demonstrated male reproductive toxicity were either of 30 days duration (Chitra et al, 1999) in pubertal rats, 21 days in weanling rats (Sinha et al, 1997b), or 7 days in prenatal rats (Sinha et al, 1997a; Sinha et al, 2001). In studies of this duration in adult rats, a relationship between time and dose can be seen. Singh and Pandey (1990) demonstrated that a dose of 7.5 mg/kg-day of endosulfan for 30 days (0.25 mg/kg-day) was required to reduce testicular testosterone, but a dose of 10 mg/kg-day could do so in only 15 days (0.67mg/kg-day).

What is OEHHA's child-specific reference dose for endosulfan?

OEHHA has selected the study by Chitra et al (1999) which demonstrated reproductive toxicity in male pubertal animals, on which to base its chRD, so the additional 10 factor that U.S. EPA maintained for its RfD, because it was based on a study in adult animals, will not be needed to protect children. OEHHA decided it was appropriate to add an uncertainty factor of 3 for the inadequacy of the database regarding neurotoxicity, as it is possible that it could be a more sensitive endpoint. The developmental endpoint of neurotoxicity has not been adequately studied and reported, and Renwick et al (2000) give inadequacy of data on a different effect than the critical effect as a reason for uncertainty in the database sufficiency. The U.S. EPA also noted this database insufficiency in the studies needed for reregistration.

The chRD for endosulfan, based on the critical effect of male reproductive toxicity in adolescent rats, using the study by Chitra et al. (1999), is 3.3×10^{-4} mg/kg-day. The significant studies with a comparison of doses and age of exposure are given in Table 1 on the next page.

Table 1. Studies on male reproductive indices

Citation	Sinha et al, 2001	Sinha et al, 1997	Chitra et al, 1999	Jaiswal et al, 2005
Dose	1 mg/kg-day in peanut oil by oral gavage to pregnant rat	2.5 mg/kg-day in peanut oil by oral gavage to 3 wk-old Druckrey rat	1 g/kg-day in groundnut oil by oral gavage to prepubertal Wistar rat	7.5 or 10 mg/kg-day in ground nut oil by oral gavage to pubertal Wistar rat
Time	GD 12 – GD 21	3-17 weeks (PND 21-90)	45 days old, 30 days of dosing	56 days old, 10 days of dosing
Plasma LDH				↑ at both doses
Testicular LDH	↑	↑		
Testicular SDH	↓	↓		
Testicular GGT		↑		
Testicular G6PDH		↑		
Testicular Lactate			↓	
Testicular Pyruvate			↓	
Testicular Ascorbate			↓	
Testicular 3B-OH steroid DH			↓	
Testicular acid and alkaline phosphatase			↓	
Testicular Protein			↑	
Testicular DNA, RNA			↓	
Testicular spermatid count	↓	↓		↓ at both doses
Epididymal spermatid count	↓	↓		↓ at both doses
Testis weight	↓	↔	↓	↓ at both doses
Epididymis weight	↓		↓	
Seminal vesicle weight	↓		↓	
Sperm Production		↓		
Plasma testosterone				↓ at both doses

Definitions from Sinha et al, 1997
LDH – a high concentration is present in the testis of newborn rats and its activity declines with the development of the testis

SDH is associated with Sertoli cell function and has been shown to exhibit an inverse relationship with spermatogenesis, i.e it is associated with pachytene stage spermatocytes that increase rapidly with the maturation of the testes.

GGT or gamma glutamyl transpeptidase is associated with Sertoli cell function and has an inverse relationship with spermatogenesis

G6PDH or glucose –6-phosphate dehydrogenase is the the marker of the functional status of Leydid cells, a site of steroid biosynthesis, it is regarded as the potential generator of NADPH which is required for hydroxylation in steroid biosynthesis, so the activity of G6PDH decreases as spermatogenesis proceeds.

Details of the Scientific Studies that OEHHA Considered in Developing a Child-Specific Reference Dose (chRD) for Endosulfan

This section of the report on endosulfan provides a more in-depth discussion of the available toxicological studies on the pesticide that relate to potentially differential effects in children compared to adults. Some information from the previous section may be repeated, but more detail is given here in order to provide the rationale for selection and rejection of studies on critical effects. Conclusions from data in this portion of the report were used in the preceding section.

OEHHA utilized three major sources of information in its investigation of endosulfan to determine if a child-specific reference dose (chRD) should be created:

1) U.S. EPA documents containing toxicity information submitted by the registrant that were reported in its Reregistration Eligibility Decision (RED) document (U.S. EPA, 2002)
2) A human study of male schoolchildren aged 10-19 years who were exposed to two-three periods during the year to endosulfan contaminated water from aerial spraying runoff from plantations on the mountains near their village in Kerala, India (Saiyed et al, 2003).
3) Animal studies in the peer-reviewed literature which reported that endosulfan affected the developing organs systems of male reproduction, neurobehavior, and immune parameters at low doses.

There were contradictions between the results of the U.S. EPA Health Effects Tests and those of the peer-reviewed literature. These will be discussed in this section.

U.S. EPA reregistration of endosulfan

Endosulfan has been undergoing comprehensive review to determine its eligibility for reregistration, in accordance with the 1988 amendments to the FIFRA, and the new safety standards of the FQPA of 1996. U.S. EPA reported in its document, *Reregistration Eligibility Decision for Endosulfan*, November 2002 (U.S. EPA, 2002), that endosulfan was eligible for reregistration if the registrant performed a series of ecotoxicology and rodent studies, the results supported registration with usage restrictions to reduce human and environmental exposure. The requested studies included a subchronic neurotoxicity study and a developmental neurotoxicity study. To mitigate dietary, worker, and ecological risks of concern for endosulfan, mitigation measures set forth in the RED included deleting use on several crops including succulent beans, succulent peas, spinach, grapes, and pecans; reducing maximum seasonal application rates for many crops; spray buffer and vegetative buffer strip requirements to protect bodies of water; Restricted Use Pesticide classification; engineering controls such as use of water soluble bags, closed mixing/loading systems, and closed cabs; cancellation of wettable powders on many crops; cancellation of aerial applications using wettable powders on many crops; and increased Restricted Entry Intervals (REIs) applicable to workers for many crops.

The U.S. EPA review for reregistration concluded that the FQPA safety factor of 10X for children should be retained for endosulfan. U.S. EPA stated that the registrant had not submitted

reliable data to address concerns or uncertainties regarding (1) evidence for increased susceptibility of young rats to toxic effects, (2) additional evidence for endocrine disruption, (3) uncertainty regarding the neuroendocrine effects in the young, and (4) the need for a developmental neurotoxicity study (DNT) (U.S. EPA, 2002). However, the DNT is designed to detect only neurobehavioral and neurotoxicity effects in the young, not the reproductive and immune system effects that are reported in the scientific literature.

Effects on male school children exposed to endosulfan

There is a recent study from the Indian National Institute of Occupational Health (Saiyed et al, 2003), which reports on endocrine effects from exposure to endosulfan on prepubertal children. Male schoolchildren 10-19 years of age in a village in Kerala, India, were exposed to endosulfan that had been aerially sprayed two to three times a year for 20 years on the cashew nut plantations on the hills above the village. Primary streams also carried soil containing endosulfan from the plantation to ponds where the children played. One hundred seventeen out of 272 male schoolchildren in the village agreed to be examined by pediatricians from the Department of Pediatrics, Kasturba Medical College, Mangalore, India. Ninety out of 135 school children in a village without endosulfan exposure were the control. Multiple regression analysis showed that the sexual maturity rating (SMR) for development of pubic hair, testes, penis, and serum testosterone level was positively related to age and negatively related to aerial exposure to endosulfan. Thus, in boys from the village of Kerala, onset of puberty or progress through puberty was delayed for their age compared to schoolchildren in the control village. Serum leutinizing hormone (LH), that stimulates testicular steroidogenesis and testosterone synthesis in the Leydig cell, initiates germ cell proliferation and puberty, levels were elevated for their age in children of the village with endosulfan sprayingindicating that a break exists between hormone stimulus and normal response . An increased number of congenital abnormalities related to testicular descent were observed in the village with endosulfan exposed children compared to the control group, although the difference was not statistically significant. The authors noted that their study was hampered by small sample size (n = 117) and the nonparticipation of 57 percent of the male schoolchildren in the endosulfan-exposed village, and 33 percent of the children in the control village.

Effects in young animals

The U.S. EPA reported that the 10X safety factor to protect children had been retained because the registrant had not submitted data to show that there was no evidence for increased susceptibility of young rats to toxic effects, nor was there evidence for endocrine disruption. In animal studies published in the peer-reviewed literature there is evidence of endocrine disruption because endosulfan has been shown to affect multiple endpoints in perinatal and adolescent animals. Endosulfan is immunotoxic, causing a decrease in immunoglobulin levels and reductions in two cell-mediated immune response assays, leukocyte migration inhibition and macrophage migration inhibition (Banerjee and Hussain, 1986; Banerjee and Hussain, 1987). Endosulfan impairs learning (Paul et al, 1994), and alters behavior influenced by neurotransmitters and neuroendocrine pathways during the prenatal and postnatal development periods in rats(Zaidi et al, 1985; Seth et al, 1986; Paul et al, 1992; Paul et al,1993). Endosulfan affects the differentiation of the testis and production of sperm in young male animals (Sinha et

al, 1997a; Chitra et al, 1999; Sinha et al, 2001). Endosulfan produced increases in pituitary and uterine weights in the two generation rat study previously mentioned (http://www.epa.gov/iris/subst/0235.htm) or (U.S. EPA, 1999a), but the results were reported as not showing reproductive toxicity.

A summary of the major factors in significant studies on the fetus and young animals exposed to endosulfan follows in Table 2:

Table 2. Summary of significant studies on young animals with endosulfan

Reference	Protocol	LOAEL/ (NOAEL)	Critical Effects
Sinha et al, 2001	Pregnant rats dosed from gestation day 12 to birth. Enzyme activities assayed on PND[a]100, or adulthood	1 mg/kg/ day, orally	Increased testicular LDH[b] and reduced testicular SDH[c]; Decreased spermatid count in testis and cauda epididymis, and weight of testis, epididymis, and seminal vesicle on postnatal day 100.
Chitra, et al, 1999.	Pubertal rats dosed for 30 days	1 mg/kg, orally	Reduced body weight and weights of testes and accessory sex organs; Decreases in testicular: lactate and pyruvate activities, DNA and RNA concentrations, 3 ß hydoxy steroid dehydrogenase enzyme activity, ascorbic acid, lysosomal acid phosphatase, and brush-border alkaline phosphatase
Sinha, et al, 1997.	Pubertal rats, dosed for 45 days – 5 days/ week until 90 days of age	2.5 mg/kg, orally	Reduced sperm count, abnormal sperm morphology; increased LDH, GGT[d] and G6PDH[e], indicators of spermatogenesis.
Jaiswal et al, 2005	Pubertal rats (56 days) dosed for 10 days	7.5 and 10 mg/kg orally	Reduced sperm count, increase in sperm abnormalities, decrease in testis weight and LDH in plasma. 5-aminosalicylic acid at 25 or 50 mg/kg daily for 10 days simultaneously or after endosulfan significantly reduced the toxicity.
Paul, et al, 1994.	Pubertal rats for 90 days	2 mg/kg-day orally	Inhibition of pole-climbing escape response to electric shock (unconditioned response) and avoidance response to buzzer (conditioned responses). Increased

Reference	Protocol	LOAEL/ (NOAEL)	Critical Effects
			5-HT[g] concentrations; learning and memory deficit; and serotonergic involvement.
Zaidi, NF et al. 1985.	0.5 - 1 mg/kg i.p. 5 days/wk from birth for 3 weeks or 5 weeks	i.p. dose 1 mg/kg-day **(**0.5 mg/ kg-day**)**	Increased serotonin binding and increased fighting behavior 8 days after cessation of treatment
Banerjee and Hussain., 1986.	Pre-pubertal rats (85-90g) fed 5,10, 20 ppm endosulfan in feed for 8 - 22 weeks Derivation of mg/kg-day dose is based on food consumption = 20 mg/day for subchronic study and average rat weight of 190mg for 8 weeks	10 ppm (0.04 mg/ kg-day), for 8 wks. is LOAEL; 5 ppm (0.02mg/ kg-day) for 8 wks is NOAEL for 8-22 week study.	Antibody titer, estimated by the indirect haemagglutination technique in tetanus toxoid stimulated rats, was significantly increased after 8 weeks at 10 and 20 ppm (0.04 mg/kg/day) and the leukocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) was significantly depressed after 8 weeks at the same dose.
Banerjee and Hussain, 1987.	Pre-pubertal rats fed 10 - 50 ppm in feed for 6 wks only	30 ppm, for 6 weeks was LOAEL, **(**10 ppm**)** for 6 weeks was NOAEL.	IgG and IgM antibody titers were significantly increased after 6 weeks at 50 ppm, and the leukocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) was significantly depressed after 6 weeks at 30 and 50 ppm. IgG & IgM[h] antibody decreased at the high dose. Leukocyte & macrophage migration inhibition depressed in mid & high doses

Reference	Protocol	LOAEL/ (NOAEL)	Critical Effects
Prenatal dosing only, so studies not considered :			
Dalsenter et al, 1999	Pregnant Wistar rats dosed from GD[f]15 to PND 22	1.5 or 3.0 mg/kg-day orally to dam	Trend for increased mortality and decreased body weight of pups at 3 mg/kg, relative testicular weight increased and mean daily sperm production decreased at puberty at 1.5 and 3.0 mg/kg-day; it did not recover in adulthood at 3 mg/kg-day.
Dalsenter et al, 2003	Pregnanat Wistar rats dosed from GD 15 to PND 22	0.5 or 1.5 mg of endosulfan/kg 21 days prior to mating, during mating, pregnancy and latation	Reproductive endpoints of the male offspring showed no decrease in sperm production, spermatid number, sperm trans, sperm morphology, and testosterone level.

[a]PND: post natal day
[b]LDH: lactate dehydrogenase; a high concentration exists in newborn rat testis and its activity declines with the development of the testis
[c]SDH: sorbitol dehydrogenase; associated with Sertoli cell function and pachtyene stge spermatocytes so it increases rapidly with the maturation of the testes
[d]GGT: gamma glutamyl transpeptidase is associated with Sertoli cell function and has an inverse relationship with spermatogenesis
[e] G6PDH: glucose-6-phosphate dehydrogenase is the the marker of the functional status of Leydig cells, a site of steroid biosynthesis, and it is regarded as the potential generator of NADPH which is required for hydroxylation in steroid biosynthesis, so the activity of G6PDH decreases as spermatogenesis proceeds.
[f]GD: gestational day
[g]5-HT: 5 hydroxytryptamine (serotonin)
[h]IgG & IgM: immunoglobulins G and M

OEHHA scientists concluded that the endosulfan studies describing male reproductive toxicity (Sinha et al, 1997, 2001; Chitra et al, 1999; ; Jaiswal et al, 2005), immunotoxicity (Banerjee and Hussain, 1986, 1987), and behavioral effects (Paul et al, 1994) from exposure during youth indicate that endosulfan fits the pattern of chemicals which have endocrine disrupting effects. Chemicals classified as endocrine disruptors typically demonstrate effects through endocrine modulation and can affect the nervous, immune, or reproductive systems subsequent to exposure at low levels during critical periods of development. Endocrine disruptors may act directly and/or indirectly on different organ systems and they may have primary effects as well as

secondary effects, depending on whether they are activating or inhibiting receptors, inhibiting the synthesis of male or female hormones, or mimicking normal hormones and altering the concentration of free and plasma protein-bound hormone (Bigsby et al, 1999). Male and female endocrine hormones affect neurological development, neuroendocrine function, and behavior, (World Health Organization (WHO) and International Programme on Chemical Safety, 2002); (http://www.who.int/pcs/emerg_site/edc/global_edc_TOC.htm) During the differentiation of reproductive organs, hormones, growth factors, and other endogenous chemical mediators regulate gene expression and direct differentiation (Cunha GR, 1992). One marked difference between exposure to endocrine disruptors during critical periods in children versus exposure during adulthood is the irreversibility of the effect during development (McLachlan JA, 1987; vom Saal FS., 1989; Greco T, 1993).

While the studies on endosulfan mentioned above indicate endocrine disruption, the dose levels in the studies on immunotoxicity (Banerjee and Hussain, 1986; 1987), in which endosulfan was administered in the feed, and the dose from i.p. administration in the study on neurobehavior (Zaidi et al, 1985) may not be precisely converted to oral or inhalation exposure. The animals in studies on male reproductive toxicity were directly administered their treatments orally. Since all studies in Table 2 have similar LOAELs and the studies in Table 1 (Chitra et al, 1999; Sinha et al, 1997, and Dalsenter et al, 1999), and the additional ones in Table 2 (Singh & Pandey, 1990 and Sinha et al, 2001) provide data which corroborate each other on male reproductive toxicity, this endpoint was chosen as the most significant.

The study by Chitra et al. (1999) used testis weight and the biochemical factors associated with spermatogenesis as indicators of normal or impaired sperm production. The study does not include the most definitive evidence: a decrease in testicular testosterone, sperm counts, and acrylic-embedded slides of developing sperm in the epididymis. However, supporting studies by Sinha et al, (1997 and 2001) reported decreased testicular and epididymal sperm counts after weanling rats were dosed with 2.5 mg/kg-day for 15 weeks, and in offspring of pregnant dams dosed with 1 mg/kg-day for 19 days, respectively. A comparison of the factors assayed in the supporting studies can be seen in Table 2.

Known spermatogenesis disruptors and discussion of effects of endosulfan on spermatogenesis

DES and zeranol, known spermatogenesis disruptors, provide positive controls for the biochemical endpoints affected by spermatogenesis disruptors. They selectively activate or inhibit orchestrated functions during development that can lead to deleterious changes in energy production and metabolism, redox status, cytochemical protection, morphogenesis, cell migration and other processes that disrupt spermatogenesis (Harris in Boekelheide et al, 1997). The studies on oral exposure of 1.0 mg/kg endosulfan to pubertal rats for 30 days, performed by Chitra et al. (1999), also demonstrated decreases in biochemical markers of energy and metabolic function of the testis, as well as decreases in the organ weights of the testis, epididymis, seminal vesicle and ventral prostrate.

The biochemical markers of energy and metabolic function of the testis are lactate, pyruvate and ascorbic acid. Endosulfan significantly decreased lactate, the major source of energy in the

testis, as well as pyruvate and ascorbic acid. Lactate is produced from pyruvate following lactate dehydrogenase A (LDH-A) action in Sertoli cells and it is transported across the plasma membrane to the germ cells by specific protein/monocarboxylate transporters (MCTs) (Halestrap and Price, 1999). Lactate is then converted into pyruvate in germ cells following lactate dehydrogenase C (LDH-C) action (Li et al, 1989). Germinal cells prefer lactate over glucose as an energy substrate (Jutte et al, 1981)(Nakamura et al, 1981), and lactate enhances in vitro survival of pachytene spermatocytes and round spermatids (Jutte et al, 1982). Lactate production in Sertoli cells has been shown to be regulated by hormones (Gnessi et al, 1997), so the alteration in the energy substrate production could be compatible with a possible testosterone deficiency (Goddard et al, 2003).

Ascorbic acid was decreased by endosulfan, and it, along with glutathione and tocopherol, are cytoprotectants to prevent oxidative stress. Ascorbic acid in semen plays an important role in prevention of oxidative damage to spermatozoa (Latchoumycandane and Mathur, 1999). Addition of ascorbic acid to in vitro rat testicular preparations increases 17β-hydroxysteroid dehydrogenase (17βHD), one of the enzymes which is critical for testosterone synthesis (Biswas et al, 1996). The same authors assayed 3β-hydroxysteroid dehydrogenase (3βHD), the other enzyme which performs an obligate step in the biosynthesis of testosterone and estrogens, as well as mineralocorticoids and glucocorticoids (Penning, 1997), and found 3βHD was decreased. Das et al. (2003) noted a similar decrease in 3βHD and 17βHD activities, testicular isomerase, plasma testosterone levels, with a decrease in counts of various germ cells after cyclophosphamide exposure. Peroxidase and catalase, antioxidant enzymes, were decreased, while malondialdehyde and conjugated dienes (evidence of oxidative stress) were elevated. All these changes were reversed by ascorbic acid co-administration. Therefore, in the Chitra et al. (1999) studies, it appears that the decrease in lactate, the source of energy for germ cells, pachytene spermatocytes, and round spermatids, and the decrease in ascorbate and 3βHD, coupled with loss of weight in testes and epidymus, indicate that a reduction in spermatogenesis likely occurred, even though this was not investigated directly by performing spermatid counts. The decreased testicular DNA and RNA reported by Chitra et al. would be expected if spermatogenesis decreased.

Acid phosphatase was also decreased by endosulfan. It is present in the acrosomes of Sertoli cells, spermatozoa, spermatocytes, and the pro-acrosome of spermatids (Males and Turkington, 1971). The specific activity of acid phosphatase normally increases 100 percent during the formation of the spermatocytes and during development of the Golgi and cap phases in the rat testis. The increase appears to reflect the continued presence of this enzyme in lysosomes of cells at stages of development, and the continued increase in acid phosphatase activity during development of the spermatid Golgi complex marks the early formation of the pro-acrosomal structures (Males and Turkington, 1971). Therefore, a significant decrease in acid phosphatase after endosulfan exposure appears to reflect decreased formation of spermatocytes, which undergo meiotic division, and spermiogenesis, or the development of spermatids into sperm.

Table 2 shows that spermatotoxicity following oral endosulfan exposure was corroborated by other studies in young rats which examined sperm counts and morphology (Sinha et al, 1997a, Sinha et al, 2001). They exposed prepubertal (21 day old) male rats from 3 weeks of age to 90 days of age to 2.5, 5.0, and 10 mg/kg endosulfan, 5 days a week (Sinha et al, 1997a), or pregnant

rats to 1 mg/kg-day endosulfan from gestational day (GD)12 to GD21 (Sinha et al, 2001). In both studies the authors measured testicular and epididymal sperm counts, organ weights, and lactate dehydrogenase (LDH) and sorbitol dehydrogenase (SDH) in the testes when the male offspring reached adulthood at 100 days of age. They found that endosulfan exposure caused a significant dose-dependent reduction in spermatid counts in both the testis and epididymis. Testis, epididymis and seminal vesicle weights were also reduced in a dose dependent manner in these adult animals. The enzymes LDH and SDH are markers of post meiotic spermatogenic cell function. In a normal testis, LDH activity decreases with the development of the testis, while SDH activity increases with the maturation of the testis (Hodgen and Sherins, 1973). If adult rats had been exposed prenatally to endosulfan, the pattern was reversed, suggesting that endosulfan interferes with the process of spermatogenesis by altering germinal epithelial cell function (Hodgen and Sherins, 1973).

Studies on adult rats also described adverse effects on spermatogenesis after endosulfan exposure, although, as expected for endocrine disrupting effects, the doses were higher than those described in younger animals (Singh and Pandey, 1990; Choudhary and Joshi, 2003). Sperm density in testis decreased significantly after 15 days of 5 mg/kg endosulfan, and sperm motility remained decreased after 30 days at the same dose (Choudhary and Joshi, 2003). The enzymes necessary for testosterone production by the testes, 3βHD and 17βHD, were decreased after 7.5 and 10 mg/kg-day of endosulfan when assayed after 30 days of dosing. Plasma LH, FSH, testosterone, and testicular testosterone were also significantly decreased (Singh and Pandey, 1990).

Dalsenter et al. (1999) treated pregnant dams orally with 1.5 and 3 mg/kg-day from GD 15 to post natal day (PND) 21 and corroborated the decrease in spermatogenesis at puberty for both doses, and at adulthood for 3 mg/kg-day. At the high dose, there was a 40 percent decrease in sperm. It is important to note that a 40 percent decrease in sperm did not produce impairment in reproductive performance after mating endosulfan-exposed male rats with control females.

Relationship of a decrease in rat sperm counts to the issue of human sperm counts

Aafjes and colleagues (1980) were interested in determining the amount of sperm reduction that is necessary to reduce fertility in rats, since laboratory rats are used in toxicity tests applied to humans. Rats with numbers of total epididymal sperm reduced from 237 million to as low as one million (4.2×10^6 +/- 3.4) from irradiation before birth were still fertile when placed in a cage with a receptive female overnight (Aafjes et al, 1980). In humans, the probability of conception in a menstrual cycle is only about 25 percent with a sperm concentration of 30-50 x 10^6/ml of ejaculate, and fertility does not increase with sperm concentrations up to 250 x 10^6/ml (Bonde et al, 1998). In the study by Bonde and colleagues (1998) on trade union members in Denmark aged 20-35 years, who attempted to achieve their first pregnancy for six menstrual cycles, 65 percent of those with a sperm count of 40 x 10^6/ml were successful, 51 percent of those with 40 x 10^6/ml or less were successful, but only 36 percent of those with sperm counts less than 20 x 10^6/ml did so. Thus, rats have much greater fertility compared to humans, and the ability of rats to procreate after exposure to environmental contaminants does not appear to be an appropriate model for maintenance of human fertility.

Questions about the stability of human sperm counts have still not been resolved. A report that worldwide sperm counts in humans were decreasing was made by collecting human data from different sources and historical time points (Carlsen et al, 1992). Reanalysis of the data indicated that many of the studies could not be compared to one another (Giwercman et al, 1993). The analysis of additional studies has demonstrated that sperm counts definitely vary by geographical location (Fisch and Goluboff, 1996). The issue of a decline at a particular location has not been resolved due to the difficulty of finding accurate historical data to compare to current data in the same locale. Swan and colleagues reported that sperm counts appear to be decreasing in the United States and Europe, but not in non-Western countries (Swan et al, 1997; Swan et al, 2000) while Fisch and colleagues (Fisch et al, 1996) reported that they were not decreasing in the United States. A recent study of sperm bank donors in different cities in the United States created more controversy because low sperm counts in Columbia, MO, compared to those in New York, NY, Minneapolis, MN, or Los Angeles, CA, were postulated to be due to the exposure to agricultural chemicals by the authors (Swan et al, 2003), because urine samples taken at the time of semen collection revealed elevated levels of the pesticides alachlor, atrazine and diazinon.

Effects of endosulfan on neurobehavior

Endosulfan caused neurobehavioral toxicity in studies by Zaidi et al, (1985) and by Seth et al, (1986). In both studies, rat pups exposed to 1 mg/kg-day endosulfan by i.p. injection from one day of age (PND1) to five weeks of age had increased serotonin levels and increased fighting behavior after receiving a foot-shock. This did not occur at a dose of 0.5 mg/kg-day. The behavior was linked to increased serotonin levels. Methysergide, a serotonin receptor blocker, administered one hour before the foot-shock test, prevented the occurrence of the fighting behavior.

Seth et al, (1986) corroborated the results of Zaidi et al, (1985) and extended the investigation, finding that endosulfan given to pups at 1 mg/kg-day i.p. increased binding of 5-hydroxytryptamine to the serotonin (5-hydroxytryptamine) receptor and decreased serotonin binding to the dopamine and benzodiazepine receptors. This changed the K_D, the dissociation constant, or the affinity of 5-hydroxytryptamine for its receptor, and the K_D and B_{max}, maximum number of binding sites, for the dopamine and benzodiazepine receptors. The decrease in binding to the dopamine receptor occurred as early as two weeks of age, but there was no change in binding of the neurotransmitters to the acetylcholine or GABA receptors. The LOAEL for i.p. injection of rat pups from PND 1 to PND 35 is 1 mg/kg-day endosulfan.

The increased sensitivity of the young to endosulfan was demonstrated by the observation that when Seth et al. (1986) treated adult animals with endosulfan, it required 3 mg/kg for 30 days (an average of 0.1 mg/kg-day) to increase binding to the serotonin receptor, whereas only 1 mg/kg for 25 days (0.04 mg/kg-day) to neonates increased binding to the serotonin receptor. Furthermore, the effect in adults was reversible when the brains of treated animals were investigated eight days after the cessation of endosulfan exposure, while it persisted in neonates when assayed at eight days. The LOAEL for neonates after i.p. dosing was 0.04 mg/kg-day.

The investigations of Paul et al, (1992; 1993; 1994) connected the increased serotonin concentrations in the brains of immature male rats, reported in the studies of Zaidi et al. (1985) and Seth et al. (1986), with disrupted learning and memory processes. Their studies were based on an association between increased serotonin levels and learning, as well as the aggressive behavior noted in the previous studies. As frequently occurs in studies on mechanism of activity, the investigators exposed immature male Wistar rats to only one oral dose of endosulfan, 2 mg/kg-day. In the conditioned avoidance response test, rats learned to climb a pole in order to escape from an electric shock just after a buzzer was sounded, so they climbed the pole at the sound of the buzzer. Rats exposed to endosulfan performed significantly worse than control animals in learning to avoid the shock, and serotonin concentrations were increased in both the cerebrum and midbrain regions. Performance on other neurobehavioral tests, such as rotorod, were used to show that the lack of avoidance behavior was not from the effects of pain from previous shocks or motor impairment (Paul et al, 1994). Rats that had learned the avoidance response after repeated trials would fail to exhibit this response when tested after 24 hours, indicating that endosulfan affected memory.

Effects of endosulfan on the immune system

A clear immunotoxic effect of endosulfan at low doses was reported in the studies by Banerjee and Hussain (1986; 1987). Endosulfan was mixed with the feed for pubertal male Wistar rats to reach concentrations of 5, 10 and 20 ppm for 8-22 weeks (Banerjee and Hussain, 1986) or 10, 30, or 50 ppm for six weeks (Banerjee and Hussain, 1987). To equate this food concentration to the dose levels in the other studies, the following methodology was used. According to U.S. EPA's Recommendations for Documentation of Biological Values for Use in Risk Assessment, (U.S. EPA, 1988) the time weighted average weight of male Wistar rats in a subchronic study is 0.217 kg for males and 0.156 kg for females, and the recommended values for food consumption is 0.02 kg/day for males and 0.016 kg/day for females. This amount of food ingestion equals exposures of 0.9 and 1.0 mg/kg-day (10 ppm), 1.8 and 2.1 mg/kg-day (20 ppm), 2.8 and 3.1 mg/kg-day (30 ppm), and 4.6 and 5.1 mg/kg-day (50 ppm). The rats were immunized by subcutaneous injection of tetanus toxoid mixed with Freund's adjuvant 20 days before terminating the exposure (Banerjee and Hussain, 1986) or after 25 days of exposure (Banerjee and Hussain, 1987). Serum immunoglobulin G (IgG) levels were significantly reduced in tetanus toxoid-stimulated rats, relative to their controls, after ingesting 10 and 20 ppm endotoxin for 12, 18 and 22 weeks (Banerjee and Hussain, 1986). Both IgG and IgM were reduced after 50 ppm endosulfan for six weeks in stimulated rats (Banerjee and Hussain, 1987). The cell-mediated immune responses of leukocyte migration inhibition (LMI) and macrophage migration inhibition (MMI) were significantly inhibited in both investigations after stimulated rats were exposed to endosulfan in their feed at 10, 20, 30, and 50 ppm (Banerjee and Hussain, 1986; Banerjee and Hussain, 1987). Thus 10 ppm, or an estimated 0.9 and 1.0 mg/kg-day for males and females, respectively, were LOAELS for immunotoxicity.

Recent studies on adult rat organ systems

Two recent studies demonstrated two heretofore unreported adverse effects on adult organ systems. Kalender and colleagues (2004a) reported that oral gavage of 1 mg/kg-day endosulfan

to adult rats for 6 weeks caused pathologic changes in adult B cells of the Langerhans islets in the rat pancreas which culminated in pycnotic nuclei and significantly increased blood glucose levels. Kalender and colleagues (2004b) reported in a separate study that 2 mg/kg-day of endosulfan to adult rats by oral gavage for 6 weeks caused cytoplasmic edema and swelling and vacuolization of mitochondria of myocardial cells. Note that the dose is given for 6 weeks, so the total dose is 84 mg/kg. There is no data in young rats or other laboratory animals.

Endosulfan's activity as an antagonist for the GABA receptor in insect brain and mammalian brain and peripheral tissues, such as testis, suggests a mode of action for sensitivity of the young

GABA is known as the predominant inhibitory neurotransmitter in mature brain, but it is an excitatory neurotransmitter during brain development which regulates morphogenesis in the immature central nervous system (Takayama and Inoue, 2004). It is excitatory in immature neurons because the concentration of chloride ion in the neuron is high, and when the $GABA_A$ receptor gate in the cell membrane opens, the chloride ion flows out and the internal concentration decreases, depolarizing the neuron and creating an action potential. In mature neurons, the concentration of chloride ion in the neuron is low, and when the $GABA_A$ receptor gate opens, the chloride ion flows into the neuron, hyperpolarizing it, so the action potential is inhibited (Takayama and Inoue, 2004).

Of special note for reports of endosulfan toxicity to the developing male reproductive system is the recent discovery that $GABA_A$ receptors are found in rat spermatozoa (Li et al, 2005) and interstitial testicular cells, most of which are testosterone-producing Leydig cells (Geigerseder et al, 2003). In addition, GABA receptors on sperm play a role in triggering the acrosome reaction whereby the sperm become active (Ritta et al, 1998; Hu and Yan, 2002; He et al, 2003). If endosulfan blocks the GABA receptors, many aspects of spermatogenesis would also be blocked.

Geirgerseder (2003) and colleagues have put forth three possibilities for GABA receptor functions in spermatogenesis:

- Regulation of endocrine function in the testis since Leydig cells produce androgens (Ritta and Calandra, 1986; 1989),
- Maturation and differentiation of germ cells because glutamate decarboxylase (GAD) mRNA has been detected in spermatids and germ cells (Persson et al, 1990; Tillakarantne et al, 1992),
- A trophic factor initiating and controlling cell proliferation and or differentiation in the interstitial compartment, as in the central nervous system (Barker et al, 1998; Lauder et al, 1998)

Blocking the GABA receptors for specific ligand-gated chloride channels by endosulfan would inhibit the testicular functions that these receptors and chloride channels perform.

Endosulfan binds to GABA receptors in insect and mammal brain and the selective toxicity ratio is 7.3 for housefly GABA receptors vs human receptors in in vitro studies (Ratra et al, 2001). The activity of endosulfan in in vitro studies on the brain suggests a mode of action for the in vivo effects of endosulfan in altering neurodevelopment and neurobehavior (Paul et al, 1994). If

the human brain is an immature one, the effects can lead to aberrant neurodevelopment. In the immature brain, binding of GABA to GABA receptors opens the chloride channel and induces efflux of chloride ions and depolarization in the immature CNS and influx and hyperpolarization in the mature CNS. If this depolarization is inhibited by endosulfan, the voltage-dependent Ca++ channels will not open to elevate cytosolic Ca++ ions and induce various steps of CNS development, such as cell proliferation, cell migration and neuronal maturation, including synaptogenesis (Takayama and Inoue, 2004).

Endosulfan uncouples oxidative phosphorylation and can induce apoptosis as another possible mode of action

Studies to investigate the mechanism of endosulfan's toxicity to the immune system utililzed a human T cell leukemic line (Kannan et al, 2000), and reported that high doses (200-1000uM) caused cell necrosis while low doses (10 – 100 uM) caused apoptosis. In both cases reactive oxygen species (ROS) were found, but only moderate levels of ROS were present at low concentrations. The lower level of oxidant stress produced a gradual, progressive loss of glutathione (GSH) and adenosine triphosphate (ATP), a reduction in the mitochondrial inner transmembrane potential, and opening of a permeability transition pore allowing the release of apoptogenic proteases to the cytosol and activation of effector caspases. This cascade leads to apoptosis, rather than to necrosis, which is seen at higher endosulfan concentrations.

The induction of apoptosis in a human T-cell leukemic line may have relevance to the in vivo findings of Banerjee and Hussain (1986, 1987) on immunotoxicity from endosulfan. Edosulfan has cuased loss of cortical lymphocytes in thymus and depressed cell mediated immunity without signs of widespread cell destruction or inflammatory changes characteristic of necrosis (Kannan et al, 2000). Apoptotic cell death does not release intracellular material into the extracellular space, so it usually does not involve an inflammatory response (Gulbins et al, 2000). Ion channels have been demonstrated to be a central element in the many triggers for apoptosis (Sah et al, 2002; Bras et al, 2005). Endosulfan has targeted the electron transport chain to interfere with mitochondrial respiration in vitro, and produce ROS (Kannan et al, 2000), which causes a significant increase in intracellular chloride that results in hyperpolarization through GABA-gated chloride channels.

Summary

Mechanism of action studies in vitro have shown that endosulfan can uncouple oxidative phosphorylaton (Kannan et al, 2000) and create ROS and oxidative stress (Sohn et al, 2004) which can produce apoptosis in immune cells (Kannan et al, 2000), testicular damage (Rao et al, 2005) and neuronal damage (Sah et al, 2002). These mode of action studies offer an explanation for the effects observed in in vivo studies of testicular damage (Sinha et al, 1997a; Chitra et al, 1999; Sinha et al, 2001), neurobehavioral disruptions (Zaidi et al, 1985; Seth et al, 1986; Paul et al, 1992;1993;1994), and immune toxicity (Banerjee and Hussain, 1986; 1987). Effects on these organ systems appear to reflect endocrine disrupting activity that occurs through antagonism of GABA receptors on chloride channels and inhibition of cellular respiration to alter differentiation during development in the young.

Recommendation

OEHHA's review of the peer-reviewed literature on endosulfan effects on male reproductive function concluded that the weight of evidence corroborated an adverse effect of endosulfan during gestation, at weaning, at puberty, and at adulthood. The doses required for an effect were higher when adults were treated. There is no way to reconcile the male reproductive deficits demonstrated in these peer-reviewed studies with the lack of reproductive effect in the two-generation study performed by the registrant (U.S. EPA, 1999) as the 1984 regulation did not require sperm counts, biochemical or hormonal assays of male reproductive function, or documentation that the desired dose was achieved.

OEHHA's proposed child-specific reference dose is based on decreased testicular, epididymal, seminal vesicle, and prostate weight and biochemical indicators of testicular toxicity in the study by Chitra et al. (1999). These researchers found that exposure to 1 mg/kg-day of endosulfan for 30 days caused a reduction in body weight and the weights of testis, epididymis, seminal vesicles, and ventral prostate, a decrease in testicular lactate and pyruvate activities, and in testicular DNA and RNA in pubertal rats. The specific activity of testicular steroidogenic enzyme, 3 β-hydroxysteroid dehydrogenase and the ascorbic acid level were decreased. These changes indicate a reduction in spermatogenesis. The studies of Sinha et al. (1997; 2001), and Dalsenter et al. (1999), which assayed spermatid counts after endosulfan exposure and found it to be depressed, corroborate the critical study by Chitra et al. (1999).

Calculation of a child-specific reference dose

Calculation of the child-specific reference dose for endosulfan is based on the following equation:

$$\text{chRD} = \frac{\text{LOAEL}}{\text{UF}} = \frac{1.0 \text{ mg/kg–day}}{3000} = 3.3\text{x}10^{-4} \text{ mg/kg–day}$$

Where,

LOAEL = Lowest Observed Adverse Effect Level (Chitra et al. 1999)

UF = Uncertainty factor of 3000 (10 for LOAEL to NOAEL, 10 for interspecies extrapolation, 10 for human variability, and 3 for inadequate database)

Comments on uncertainty factors

U.S. EPA noted that the Rat Developmental Neurotoxicity Study constituted a data gap when the RfD was established. The U.S. EPA review for reregistration concluded that the FQPA Safety Factor of 10X should be retained for endosulfan as there were no reliable data available to address concerns or uncertainties regarding (1) evidence for increased susceptibility of young rats, (2) additional evidence for endocrine disruption, (3) uncertainty regarding the neuroendocrine effects in the young, and (4) the need for a developmental neurotoxicity study (DNT) (U.S. EPA, 2002). OEHHA staff has selected studies in pubertal animals on which to base its chRD, so an additional 10X factor will not be needed to protect children.

OEHHA decided it was appropriate to add an uncertainty factor of 3 for database inadequacy in regard to neurotoxicity, immunotoxicity, length of studies, pathologic changes in adult B cells of the Langerhans islets in the rat pancreas islets (Kalender et al, 2004a) and cytoplasmic edema and swelling and vacuolization of mitochondria of myocardial cells (Kalender et al, 2004b). First, the investigations of Paul et al, (1992; 1993; 1994) connected increased serotonin concentrations in the brains of immature male rats [reported in the studies of Zaidi et al. (1985) and Seth et al. (1986)] with disrupted learning and memory processes, as well as aggressive behavior, but a single dose was used to produce the frank effect so there is no information on the lowest LOAEL or a NOAEL. U.S. EPA also noted missing neurotoxicity studies, kept a 10X safety factor, and made satisfactory findings on neurotoxicity a requirement for reregistration. Second, the immunotoxicity studies of Banerjee and Hussain (1986; 1987) on prepubertal rats were feeding studies, but the amount of feed eaten/day and body weights during the 6-8 week studies are not provided. The use of default data from “Recommendations for and

Documentation of Biological Values for use in Risk Assessment" (U.S. EPA, 1988) suggests that a very low dose causes significant decreases in cell mediated immunity (CMI).

Third, the studies demonstrated male reproductive toxicity in young rats were of short duration: 30 days (Chitra et al, 1999) in pubertal rats, 21 days in weanling rats (Sinha et al, 1997), or 7 days prenatally (Sinha et al, 1997; Sinha et al, 2001). Furthermore, as Table 3 below demonstrates, studies showing reproductive toxicity (Singh and Pandy (1990), neurotoxicity (Zaidi et al,1985) and immunotoxicity (Banerjee and Hussain, 1986; 1987) demonstrated that the dose may need to be given for a sufficient duration for the adverse effect to be expressed.

Table 3: Studies indicating a data gap in understanding endosulfan's potential toxicity

STUDY	ENDOSULFAN DOSE	TIME	OUTCOME
Singh and Pandey, 1990	7.5 mg/kg-day	15 days	No effect on synthesis of testicular testosterone
	7.5 mg/kg-day	30 days	Reduced synthesis of testicular testosterone
	10 mg/kg-day	15 days	Reduced synthesis of testicular testosterone
Zaidi et al, 1985	25 doses of 0.5 mg/kg, ip	5 weeks	No increase in fighting behavior
	25 doses of 1.0 mg/kg, ip	5 weeks	Fighting behavior increased after foot shock
	25 doses of 1.0 mg/kg, ip and serotonin receptor blocker 1 hr before assay	5 weeks	No increase in fighting behavior
Zaidi et al, 1985	Newborn rats given 25 doses of 0.5 mg/kg, i.p	3 weeks	No effect on serotonin binding
	Newborn rats given 25 doses of 0.5 g/kg, i.p	5 weeks	No effect on serotonin binding
	Newborn rats given 25 doses of 1.0 mg/kg, i.p	3 weeks	No effect on serotonin binding
	Newborn rats given 25 doses of 1.0 mg/kg i.p	5 weeks	Increased serotonin binding to cell membranes in prefrontal cortex
Banerjee and Hussain, 1986	10 ppm in feed	8 weeks	Significant decrease in cell mediated immune response in rats immunized with tetanus toxoid

STUDY	ENDOSULFAN DOSE	TIME	OUTCOME
Banerjee and Hussain, 1987	10 ppm in feed	6 weeks	No effect on cell mediated immune response
	30 ppm in feed	6 weeks	Significant decrease in cell mediated immune response in rats immunized with tetanus toxoid

The chRD is used in situations where children will likely be exposed during the school day for several years. The initial biochemical or cellular cause of the disruption to spermatogenesis in the 30-day study by Chitra et al (1999), or the supporting studies, has not been identified; so it is not known if there is a short or long critical window of vulnerability or what the threshold dose over a month, year, or all of adolescence would disrupt spermatogenesis in adolescent male humans.

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